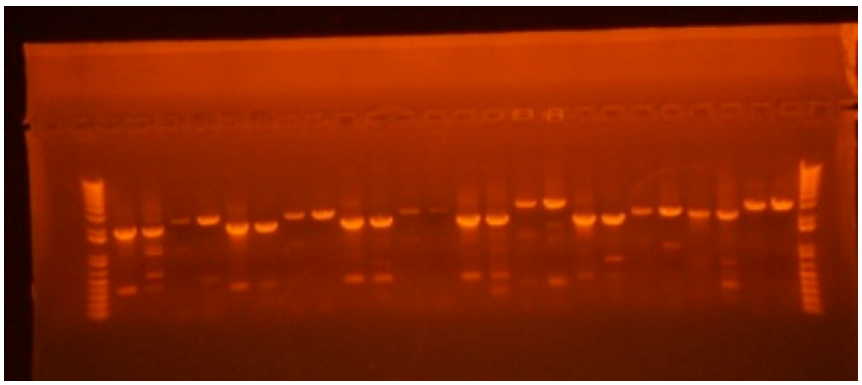
Jason Cham start 7/01/11

From AndersonLab wiki

Contents

Jcham 04:36, 01 July 2011 (PDT)

Splitting PCRs



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1-2:bjc005-jc1-L xpand/phusion
lane 3-4:bjc005-jc1-R xpand/phusion
lane 5-6:bjc005-jc2-L xpand/phusion
lane 7-8:bjc005-jc2-R xpand/phusion
lane 9-10:bjc005-jc3-L xpand/phusion
lane 11-12:bjc005-jc3-R xpand/phusion
lane 13-14:1832-np1-L xpand/phusion
lane 15-16:1832-np1-R xpand/phusion
lane 17-18:1839-np2-L xpand/phusion
lane 19-20:1839-np2-R xpand/phusion
lane 21-22:1832-xl1-L xpand/phusion
lane 23-24:1832-xl1-R xpand/phusion
```

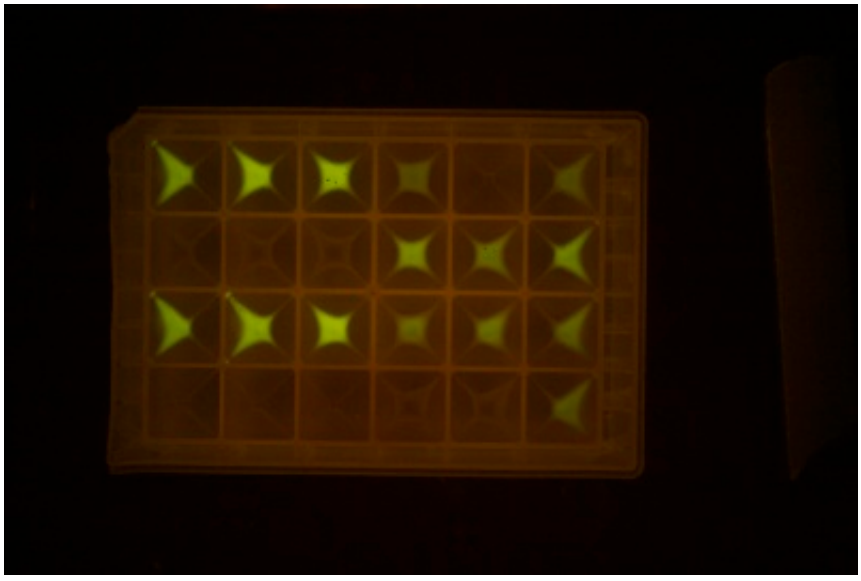
digest, gel purify, ligate with appropriate stuffers, transform into jtk155

Jcham 03:36, 02 July 2011 (PDT)

- transform fabB, gyrA, hisS, mqsA, tadA, yeaZ, orn into bss55.
- pick split colonies.
- miniprep splits

Jcham 11:23, 05 July 2011 (PDT)

- transform bss into mc1061 cells to make comp cells in case the ones already made are contaminated.
- test current comp cells by transforming water, +ctrl, and -ctrl into bss-52 cells.
- innoculate seven tests of new template proteins
- send all splits for sequencing.



```
well 1-3 gyrA
well 4-6 fabB
well 7-9 mqsA
well 10-12 hisS
well 13-15 yeaZ
well 16-18 tadA
well 22-24 orn
```

Tecan Results

1219.519	961.8832	1247.926	3852.788	4751.838	4039.971	fabB	gyrA
3504.283	2642.521	3406.272	117.411	142.0642	124.7761	hisS	mqsA
2084.769	2028.841	1796.482	4471.159	4561.216	3917.436	tadA	yeaZ
1254.555	112.302	116.1651	6056.997	105.6092	6249.237	orn	`+/-/+

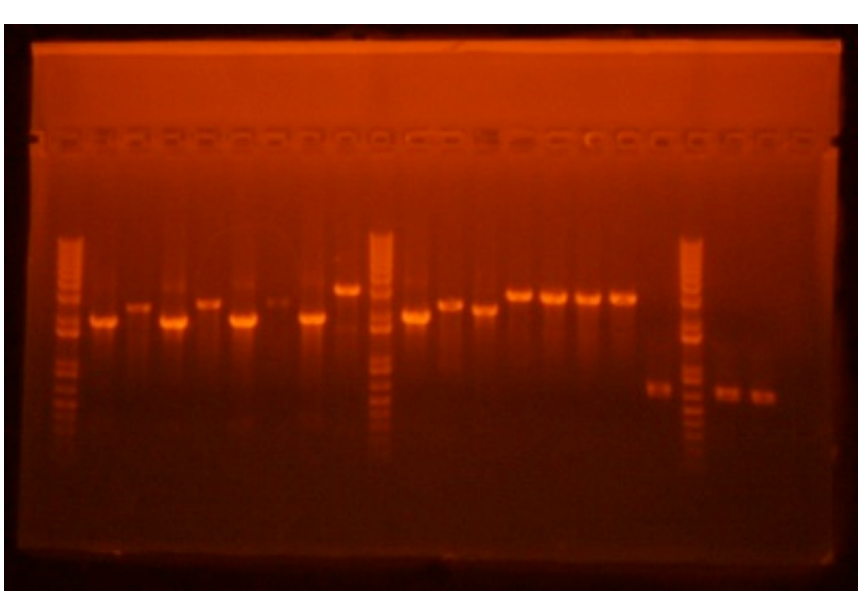
We are going to go with gyrA, hisS, tadA, and yeaZ to make further libraries.

Jcham 4:36, 06 July 2011 (PDT)

- The sequencing for the splits turned out bad.
- I will redo the digest from the pcr products.

Digest: procedure in order 4uL H20, 1 uL NEB2, 0.5uL KpnI/SpeI, 4uL part 1 hr 30 min.

Digestion Gel KpnI/SpeI:



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: bjc005-jc1L
lane 2: bjc005-jc1R
lane 3: bjc005-jc2L
lane 4: bjc005-jc2R
lane 5: bjc005-jc3L
lane 6: bjc005-jc3R
lane 7: 1832-np1L
lane 8: 1832-np1R
lane 9: 1839-np2L
lane 10: 1839-np2R
lane 11: 1832-xl1L
lane 12: 1832-xl1R
lane 13: r6k+kan
lane 14: r6k+kan
lane 15: r6k+kan
lane 16: r6k
lane 17: r6k
lane 18: r6k
```

Gel Purify

- cut bands out and add 600 uL ADB and melt gels in 55 degrees.
- put melted gel into zymo column, spin down 30 sec.
- pour out flow through
- 250 uL PE wash buffer, centrifuge 30 sec
- 250 uL PE wash buffer, centrifuge 30 sec
- discard flow through
- dry for 90 sec
- new labeled tubes, elute with 10 uL water.

Ligation: 4.3 uL part 1, 4.3 uL part 2, 1 uL ligase buffer, 0.5 uL ligase half an hour

Transformation

- jtk155 add 30 uL KCM
- 70 uL cells into ligation mix
- 10 min on ice, 1:30 min 42 degree C, 1 min on ice
- recover with 2YT for 1 hr.

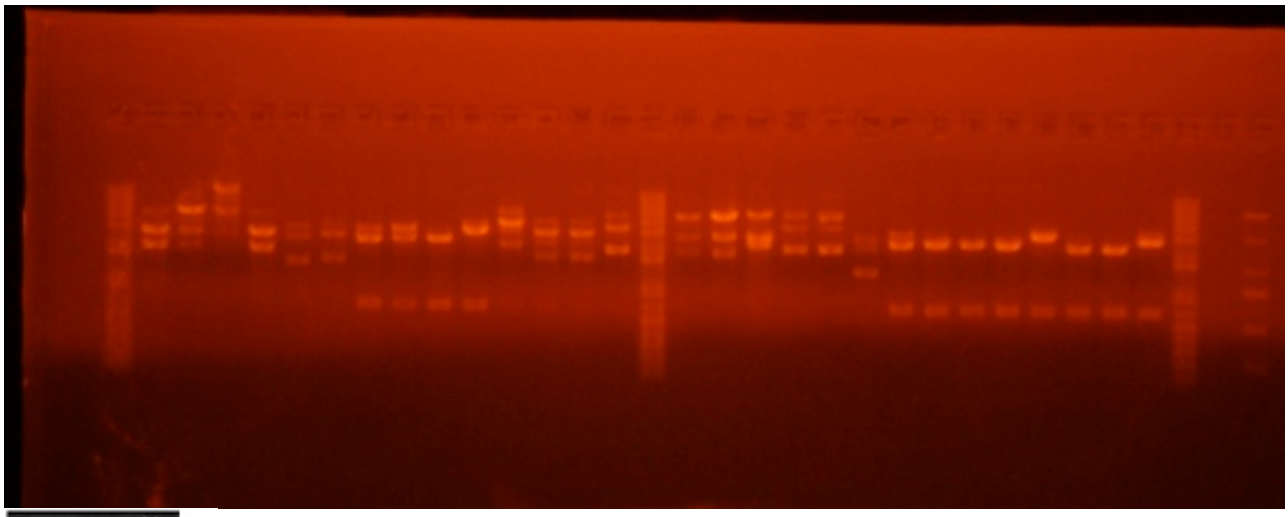
Plate lefty on kan, righty on spec

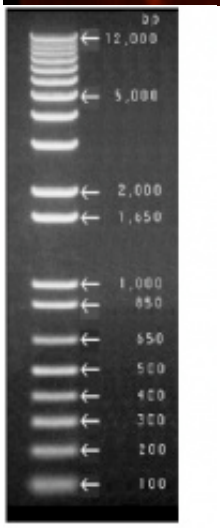
Jcham 11:00, 07 July 2011 (PDT)

- pick colonies, will mini prep tomorrow

Jcham 1:17, 08 July 2011 (PDT)

- mini prep all of the splits.
- digest map of the miniprep products



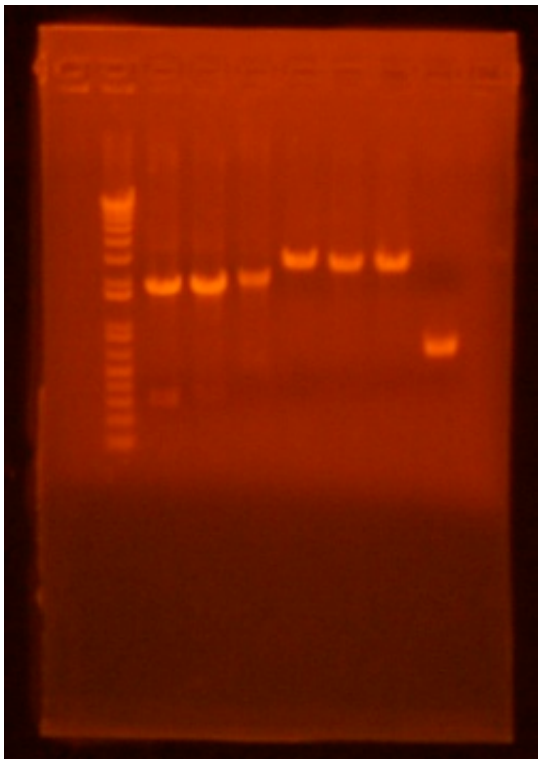


1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1:jc1L
lane 2:jc2L
lane 3:jc3L
lane 4:np1L
lane 5:np2L
lane 6:xl1L
lane 7:jc1R
lane 8:jc2R
lane 9:jc2R
lane 10:jc3R
lane 11:np1R
lane 12:np2R
lane 13:np2R
lane 14:xl1R
repeat
```

Jcham 4:22, 11 July 2011 (PDT)

- digest jc3L, np2L



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

- gel purify
- ligate with r6k+kan
- transform into jtk155
- recover
- plate on Kan

- sequence jc1L, jc2L, np1L with ca998
- set up gold reaction to test library splits. JC1 JC2 NP1

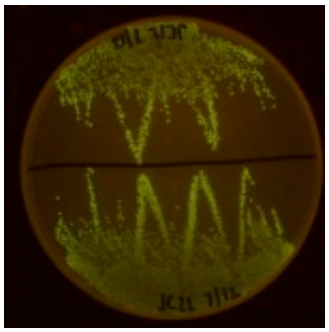
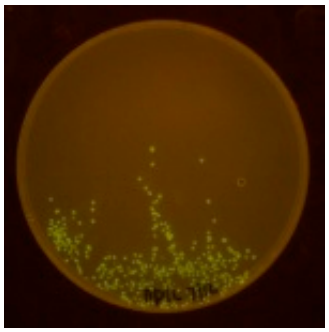
Jcham 1:10, 12 July 2011 (PDT)

- zymo clean up gold reaction, elute in 12 uL
- transform into bss52
- recover in 2yt for 1 hr
- plate on CAS

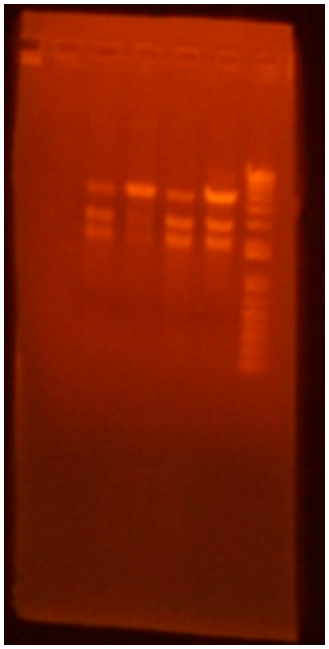
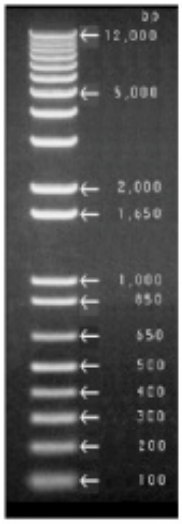
- pick JC3L, NP2L colonies into Kan+LB

Jcham 2:42, 13 July 2011 (PDT)

- The test gold reactions on the library splits worked. The plates for jc1, jc2, np1 were green.

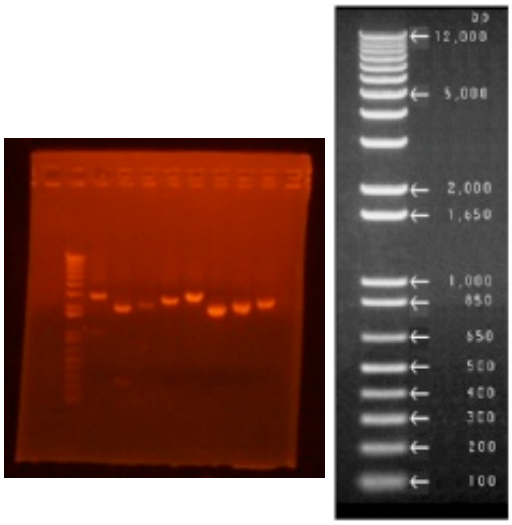
- I miniprep'ed jc3L, np2L
- The digest mapped decently.
- I will sequence them with ca998 and ca1786

1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1:jc2L 1 KpnI/speI
lane 2:jc2L 2 kpnI/speI
lane 3:np2L 1 kpnI/speI
lane 4:np2L 2 kpnI/speI
```

I set up pcr's for mukF interface libraries 1 and 2

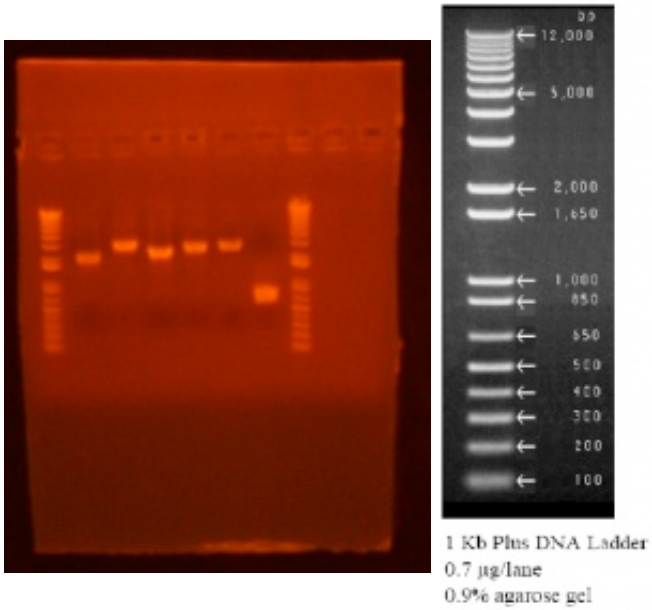


1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: xl1R
lane 2: xl1L
lane 3: xl2L
lane 4: xl2R
```

Jcham 11:08, 14 July 2011 (PDT)

- set up digest for mukF interface xl1 and xl2



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

```
lane 1: xl1L kpnI/speI
lane 2: xl1R kpnI/speI
lane 3: xl2L kpnI/ speI
lane 4: xl2R kpnI/ speI
lane 5: r6k + kan
lane 6: r6k
```

- ligate lefty with r6k+kan and ligate righty with r6k

- transform into jtk155 pir
- recover in 2YT for 1hr.
- plate lefty on kan, plate righty on spec

- sequencing came back for the previous two splits jc2L and np2L. The sequences are good.
- ran a 15ml scale one pot reaction (Digestion and ligation) used gold program on PCR machine

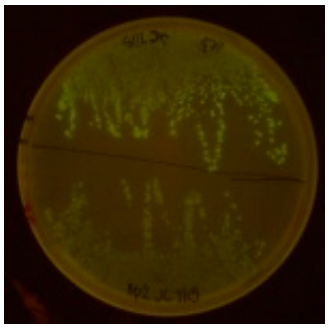
```
1. .3 ul BseRI, .3 ul ligase, 1.5ul T4 DNA ligase buffer, 1.1ul 1778, 1.1 ul 1784, 11ul h20
```

Jcham 11:48, 15 July 2011 (PDT)

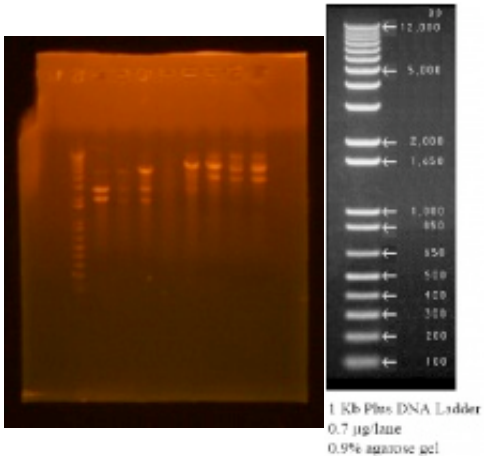
- zymo clean gold reaction on jc3 and np2 library
- 45 uL ADB, spin for 30 sec, 200 uL wash buffer, spin, 200 uL wash buffer, dry for 90 sec, elute with 15 uL.
- transform into bss52

Jcham 11:32, 18 July 2011 (PDT)

- The gold reaction worked for jc3 and np2 library



- miniprepped the cultures of xl1 and xl2 libraries.
- set up test digest with kpnI/SpeI



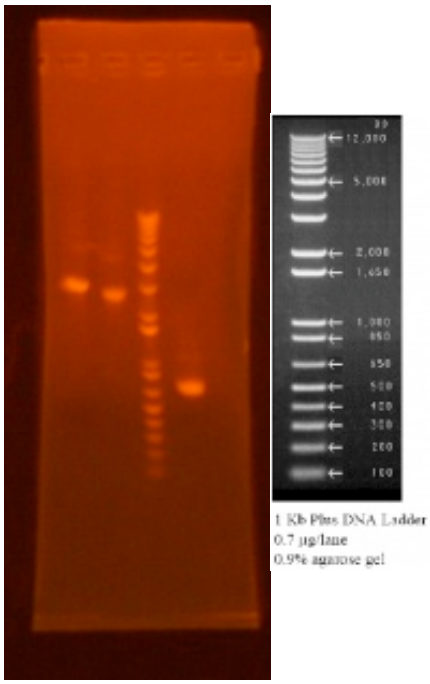
```
KpnI/SpeI Map Digest
lane 1:xl1L 1
lane 2:xl1L 2
lane 3:xl2L 1
lane 4:xl2L 2
lane 5:xl1R 1
lane 6:xl1R 2
lane 7:xl2R 1
lane 8:xl2R 2
```

- send xl1L and xl2L out for sequencing
- redo xl1R and xl2R tomorrow

Jcham 12:27, 19 July 2011 (PDT)

- The sequences for xl1L and xl2L came back fine.

- digest xl1R and xl2R




```
lane 1: xl1R kpnI/speI
lane 2: xl2R kpnI/speI
lane 3: r6k kpnI/speI
```

- gel purify
- ligate to r6k stuffer
- recover in 2yt for 1 hr
- plate on spec

Jcham 4:20, 20 July 2011 (PDT)

- pick colonies on xl1R and xl2R

Jcham 2:21, 21 July 2011 (PDT)

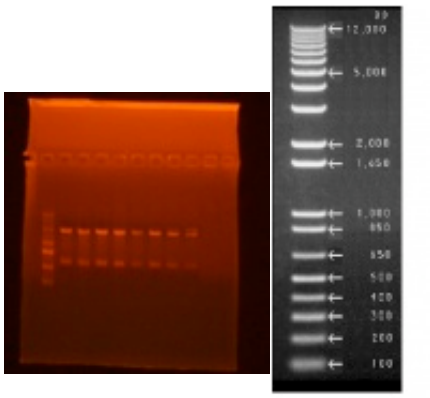
- miniprepped xl1R and x12R
- map digested. xl1R worked fine. xl2R needs to be redone
- religate r6k with xl2R fragment.
- transform into jtk155

Jcham 12:00, 25 July 2011 (PDT)

- pick xl2R colonies

Jcham 7:00, 26 July 2011 (PDT)

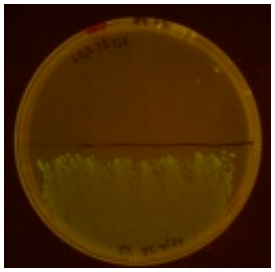
- help miriam with EIPCR, gel purify, gold reaction
- sequence xl2R
- set up gold reaction for xl1 and xl2
- map digest of xl2R



1 Kb Plus DNA Ladder
0.7 µg/lane
0.9% agarose gel

Jcham 4:00, 27 July 2011 (PDT)

results for the test gold reaction on xl1 and xl2



xl1's gold reaction worked, but xl2 did not.Sequencing says that both parts of xl2 are correct. I will do the gold reaction again to see if the splits can be put together again.

reaction again to see if the splits can be put together again.